

Bioassay, carcinogenesis and tissue culture

#159R3

Committee:  
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TOBACCO INDUSTRY RESEARCH COMMITTEE  
150 East Forty Second Street  
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Activated: 9/1/57  
Renewed: 9/1/58  
Extended to: 4/1/60

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Application for Renewal of Research  
Grant

Date: November 8, 1960

1. Name of Investigator: Cecilia Leuchtenberger, Ph.D.
2. Title: Senior Biologist and Cytochemist
3. Institution & Address: Children's Cancer Research Foundation  
35 Binney Street  
Boston 15, Mass.

Address after April 1, 1961:

Inst. f. Allgemeine Botanik  
d. Eidg. Techn. Hochschule  
Zuerich, Switzerland  
Director, Prof. A. Frey-Wyssling

4. Project or Subject:

The interrelation between influenza virus infections and exposure to cigarette smoke in the development of pulmonary and of bronchial lesions in mice.

(A correlated histological, cytological and cytochemical study of the tracheobronchial tree and lungs.)

5. Detailed Plan of Procedure:

The purpose of this project is the continuation and extension of the study of the sequence of events occurring in the tracheobronchial tree and lungs of mice exposed to cigarette smoke. Particular attention will be focused on the possible role of influenza virus infections in the production of bronchial and pulmonary lesions. Correlated histological, cytological and cytochemical investigations will be carried out and combined with the application of newer quantitative cytochemical techniques, such as microspectrophotometry, interference and fluorescence microscopy. It is felt that these methods will be of great help when attempting to explore the interrelationship between virus infections and exposure to cigarette smoke in the development of bronchial and pulmonary lesions in mice.

Results of previous studies done in our laboratory on the project, which are concerned with the histopathological, cytopathological and cytochemical changes in the tracheobronchial tree and lungs of mice after exposure to cigarette smoke, have been reported at National and International Congresses and have been published under the titles:

1003541401

- I. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Bronchitis with Atypical Epithelial Changes in  
Mice Exposed to Cigarette Smoke  
Cancer, Vol. 11, No. 3, May-June, 1958

- II. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE SEQUENCE OF EVENTS IN THE BRONCHIAL EPITHELIUM FROM MICE EXPOSED TO CIGARETTE SMOKE

Acta Union Internationale contre le Cancer, Vol. XV, Nos. 3-4, 1959

- III. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Varying Responses of Major Bronchi to Cigarette Smoke,  
Absence of Bronchogenic Carcinoma after Prolonged Exposure,  
and Disappearance of Bronchial Lesions after Cessation of  
Exposure  
Cancer, Vol. 13, No. 4, 1960

- IV. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Unaltered Incidence of Grossly Visible Adenomatous Lung Tumors in Female CF<sub>1</sub> Mice after Prolonged Exposure to Cigarette Smoke  
Cancer, Vol. 13, No. 5, 1960

On the basis of the studies, published under I-IV, it appeared that in cases in which a harmful effect on the tracheobronchial tree has been observed after exposure of mice to cigarette smoke, the cigarette smoke may not be solely responsible, but that there may be other elements within the host which may render the tissue susceptible to smoke. The concept that the tracheobronchial tree may, indeed, need a "special conditioning" for the injurious action of the cigarette smoke is supported by our data on mice - that the difference in response and resistance of the bronchi to cigarette smoke is independent of dose and length of exposure.

While it is impossible, at present, to relate any particular factor or factors of the host to the resistance or susceptibility of the tracheobronchial tree to cigarette smoke, the investigation of agents possibly carried by the host, which may contribute to the injury of the bronchi, would seem of great interest. Among the many possible host factors, viruses deserve special consideration - the more so since they occur with a certain frequency in mice and are capable of producing respiratory lesions.

It is well known that mice may be carriers of latent viruses which, under certain circumstances, may provoke alterations. In view of the increasing recognition of the dual role of viruses as either cytopathic agents or stimulators of cell proliferation, which may even lead to tumor formation, the investigation of viruses in combination with exposure to cigarette smoke seems to us particularly worthwhile.

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At present we are engaged in studies concerned with the question of whether a mouse adapted influenza-virus will alter the effect of exposure to cigarette smoke on the tracheobronchial tree and lungs of mice.

For this purpose, mice were divided into 5 groups as follows:

Group 1, consists of non-exposed controls

Group 2, consists of mice infected intranasally with influenza virus

Group 3, consists of mice exposed to cigarette smoke

Group 4, consists of mice exposed to cigarette smoke followed by intranasal influenza virus infection

Group 5, consists of mice infected intranasally with influenza virus followed by exposure to cigarette smoke.

In view of the importance of long range studies, mice were infected with influenza virus in such a manner that survival of these animals was assured.

During the remaining time in Boston, these mice will be sacrificed at certain intervals and all the organs necessary for this study processed for the combined morphological and cytochemical examination. This material will be taken to the Institute for Allgemeine Botanik (Director - Prof. Dr. A. Frey-Wyssling) at the Eidgen. Techn. Hochschule in Zuerich, Switzerland, where it will be further prepared in order to carry out the proposed combined morphological and cytochemical investigations at the department of cytochemistry (Prof. F. Ruch) at the Institute in Zuerich.

6. Budget Plan:

\* see under 10

Salaries	\$ 22,500
Expendable Supplies	1,500
Permanent Equipment	-----
Overhead (none)	-----
Other	-----
	-----
	\$24,000

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7. Anticipated Duration of Work: 1 to 2 years

8. Facilities and Staff Available: The Institute for Allgemeine Botanik at the Technische Hochschule in Zuerich, Switzerland, has a special department of cytochemistry (Prof. F. Ruch) with all facilities for histo-and cytopathological work. This laboratory is also equipped with numerous modern optical instruments including special scanning microspectrophotometer and microinterferometer which will be completely at our disposal. Prof. F. Ruch, who designed some of these instruments, is greatly interested to collaborate with us in applying these special methods and instruments for the study of the chemical alterations at the single cell level after virus infections and exposure to cigarette smoke.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects  
and other sources of supply):

Explanation of Salaries:

Senior investigator \$11,000 (present salary)

Part-time Pathologist 8,000

Laboratory assistant 3,500

The \$1500 will be needed for chemicals, glassware, slides, enzymes etc.

/s/ Cecilia Leuchtenberger  
Director of Project

Business Officer of the Institution

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